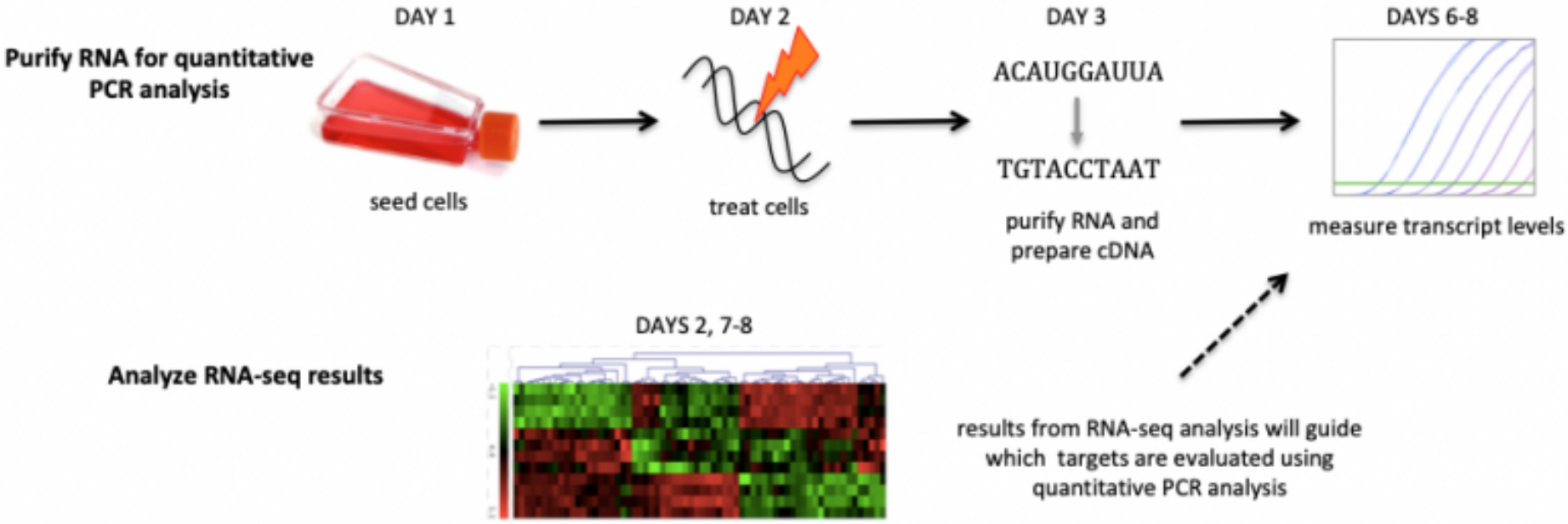
M2D2: Induce DNA damage for RNA purification

1. Comm lab workshop
2. Prelab
3. ½ class to TC to etoposide treat cells
4. ½ work on Rstudio.cloud Intro to Clustering and Exercise #2

For JC HW slide: if you like your slide, great! If you would change it after Comm lab, submit updated version to Stellar by 10m TONIGHT

Mod2: Experimental overview

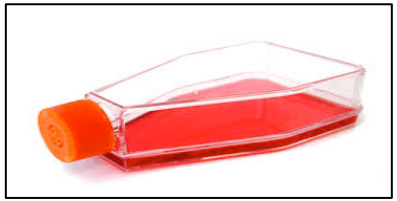


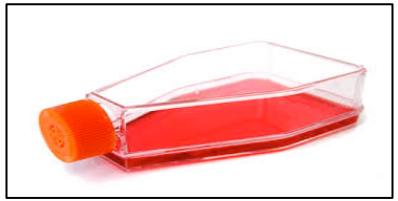
Mod2 major assignments

- **Research Article (20%)**
  - individual, submit on Stellar
  - due Saturday April 19th at 10pm
  - format: word document, figures can be submitted separately
- **Journal Club Presentation (15%)**
  - individual presentation
  - presentation slides due on Stellar prior to presentation
  - format: powerpoint, keynote, or google slides
- Lab quizzes (5%) M2D3, M2D8
- Blog (5%), 3 posts for full credit
  - March 16 at 10 pm, April 20 at 10 pm, May 9 at 10 pm, May 12 at 10pm

Treat DLD-1 cells with etoposide

DLD-1





+ etoposide
(60 min)

replace all media with fresh (no drug added) media

M2D3: extract RNA

(~48 hours after DNA damage)

100 μM in 10mls

Stock etoposide 100mM

C1*V1=C2*V2

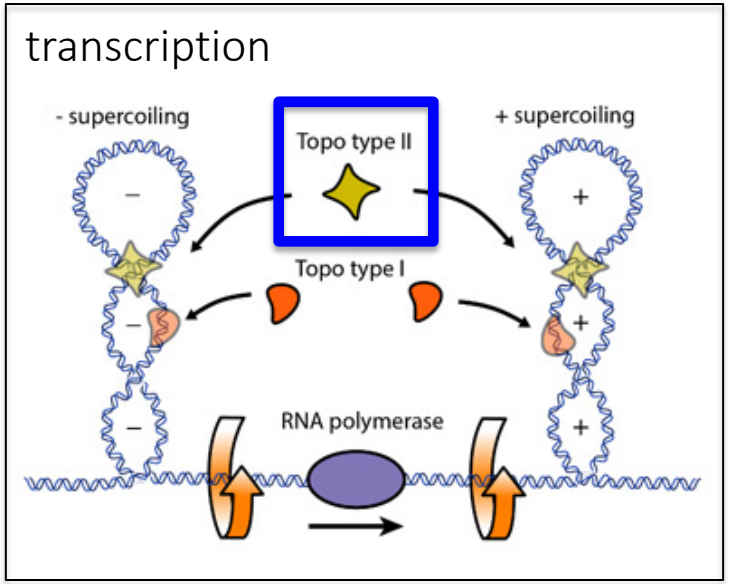
(100mM)(X) = (0.1mM)

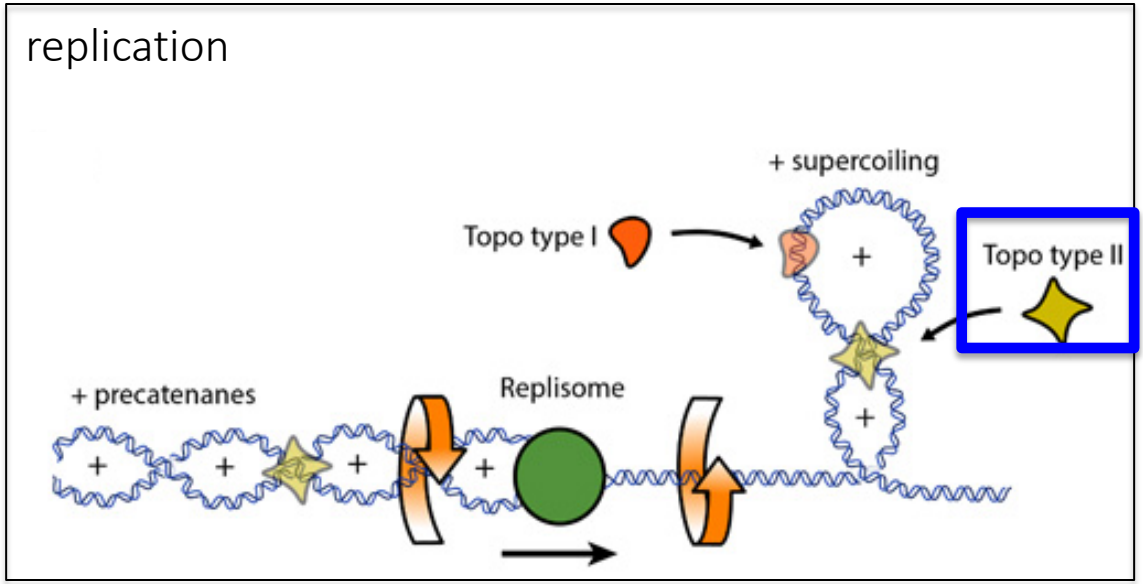
(10,000μl)

10 μl

RNA Transcription and DNA Replication cause DNA supercoiling

Normal function: Topo Type II (topoisomerase II enzyme) relieves supercoil tangles

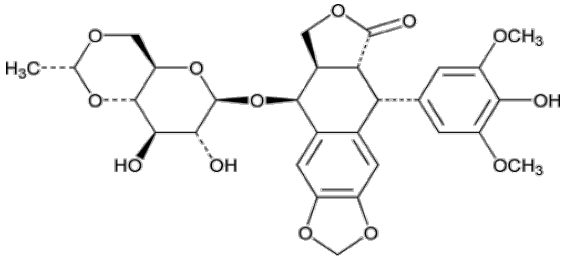




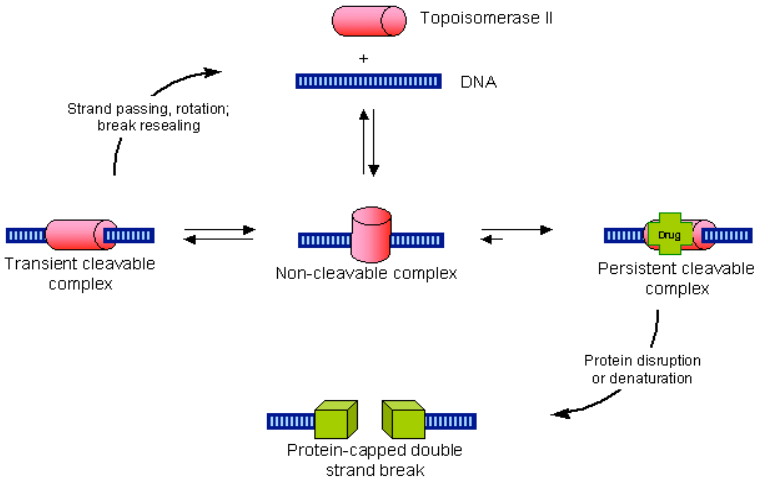
Ma, J. & Wang, M.D. Biophys Rev (2016) 8(Suppl 1): 75. https://doi.org/10.1007/s12551-016-0215-9

Etoposide is a drug/chemotherapy that causes DNA double strand breaks

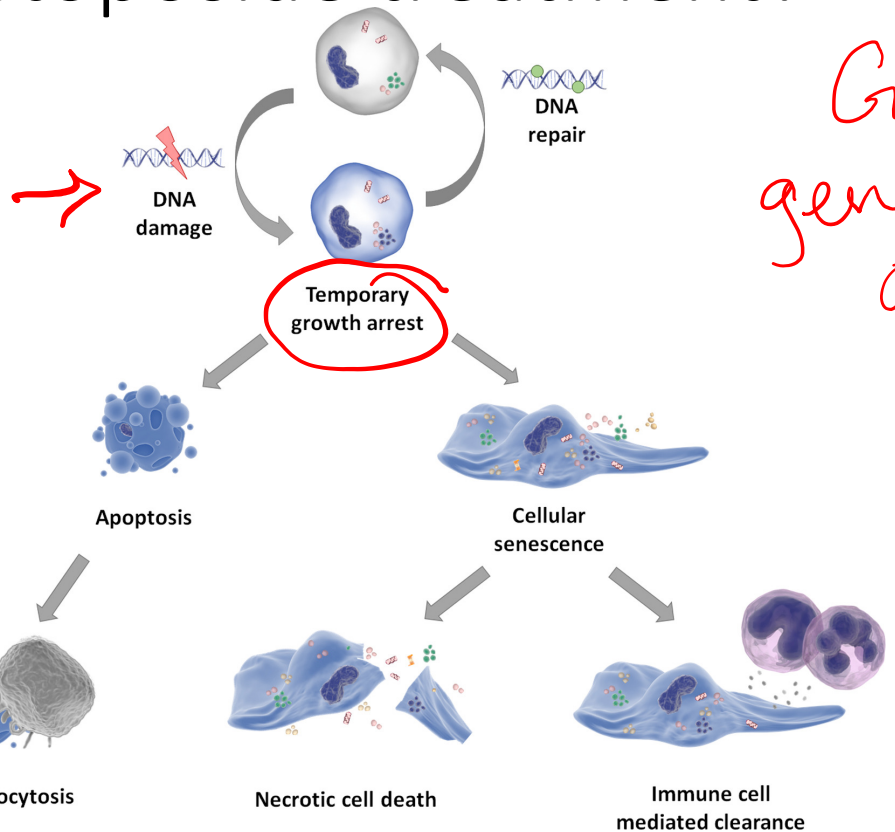
- mechanism of action: forms a ternary complex with DNA and topoisomerase II enzyme and prevents re-ligation of the DNA strands = double strand DNA strand break
- cancer cells (quickly dividing cells) rely on topoisomerase II more than normal cells and therefore have more double strand DNA breaks when treated with etoposide



Etoposide structure



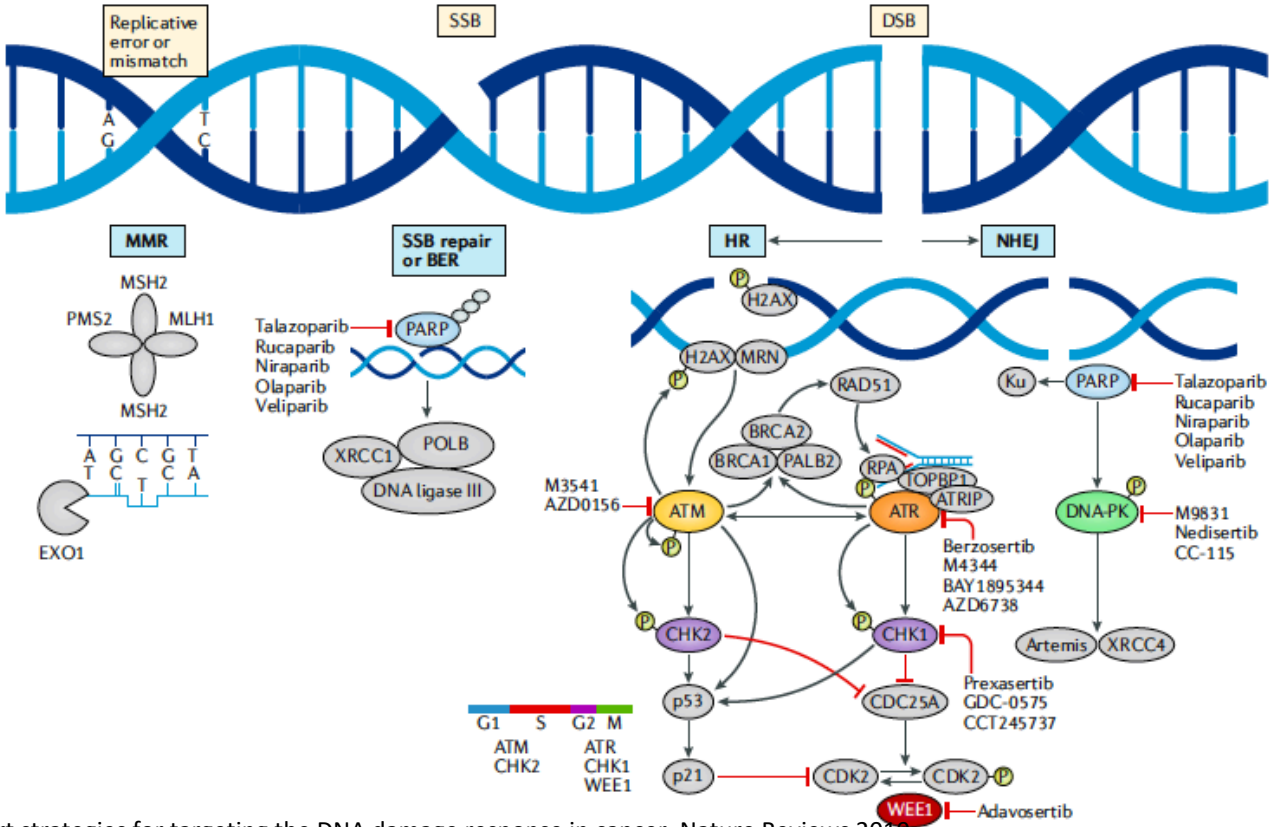
What cellular functions change upon etoposide treatment?



GO terms
gene ontology

Soto Gamez et al. Regulation of Survival Networks in Senescent Cells: From Mechanisms to Interventions. JMB July 2019

What genes are differentially expressed in response to etoposide treatment?



Pilié et al. State- of-the- art strategies for targeting the DNA damage response in cancer. Nature Reviews 2019.

R analysis in benchling notebook

Reminder: each lab day with a R exercise, Joe will check your progress in benchling before you leave

Today for Intro to clustering :

- ❑ Hierarchical Clustering heatmap
- ❑ K-means Clustering color plot
- ❑ Principal Components Analysis: save all heatmaps and PCA plots
  - Answer all questions at the top of page 2 in the pdf

Today for exercise #2:

- ❑ Ok to come back to a lot of the thought questions on your own time
- ❑ Top downregulated and **upregulated** gene ontology terms from the DLD-1/DLD-1 etoposide treated cells

Today in lab:

1. Tissue Culture (TC)
   - 1st : Red, Orange, Yellow, & Green & Blue
   - 2nd: Pink, Purple, White, & Silver
   - Protocols printed for TC use, no need to move laptops etc.

   ➢ Do not wear PPE in or out of TC room
2. Work on exercise #2 in Rstudio.cloud
3. Read your Journal Club paper

- Homework due Friday, M2D3 – by the end of class
  - Figure/title/caption, Results and Discussion

M2D3HW

- Figure= the top five up and down gene ontology (GO) terms from DLD-1 +/- etoposide
- Figure must include a title and caption
- associated results and discussion **paragraphs**
  - **Mod2 results text will not include interpretation of the data shown in the figure**
  - Separate discussion section associated with figure with interpretation
- review guidelines on the wiki homework tab!!

RESULTS

1. What was the overall goal of these data?
   - State concisely as an introductory sentence.
2. If applicable, what was the result of your control?
   - Was it expected?
3. What was your result?
   - Was it expected?
4. What does this motivate you to do next?
   - Specifically, what experiment follows?

DISCUSSION

1. What evidence do you have that your result is correct or incorrect?
   - How do your controls support your data?
2. In sum, what do your data suggest or indicate?
   - Do your data support your hypothesis? Why?
3. What does this motivate you to do next?
   - Specifically, what is the next research question?